Molting and *Salmonella Enterica* Serovar Enteritidis Infection: The Problem and Some Solutions

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ABSTRACT Induced molting is an important economic tool used by the egg industry to recycle an aging layer flock. It is estimated that approximately 70% of the flocks nationwide and almost 100% in California are molted annually. Considering that there are approximately 240 million hens in production in the U.S., a rough estimate of the numbers of hens molted every year would be between 144 and 168 million birds, a substantial number. There are many methods to induce molt, but feed removal until hens lose a specific weight is the most prevalent molt strategy in the U.S. However, experimental studies in our laboratory have shown that induced molting via feed removal depresses the immune system of hens and exacerbates a *Salmonella enteritidis* (SE) problem in a simulated flock situation. Molted hens excreted significantly higher SE numbers in the feces, had higher numbers of SE in internal organs, and exhibited more intestinal inflammation. Molted hens were 100- to 1,000-fold more susceptible to infection by SE and therefore more readily transmitted the organism to uninfected hens in neighboring cages. With the problems identified, solutions were sought, and several were successful in ameliorating the SE issue. Antibiotic therapy, vaccination, and use of low-energy, low-calcium diets to molt hens all dramatically decreased SE shedding during molt. All of the solutions provide the producer with many potential solutions to the SE food safety issue and still allow them to recycle their hens.

(*Key words*: food safety, *Salmonella enterica* serovar Enteritidis, molting, stress)

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INTRODUCTION

The commercial table egg business remains a major economic presence in the U.S. To ensure the economic viability of the industry, a number of management tools are implemented to maximize the effective production capability of the laying flocks. Vaccination, biosecurity, medicated feed, and increased stocking densities, to name a few, are used to improve the health of birds and optimize the useable space within a house. However, these tools can have little impact on an important determinant of productivity—age. As a laying flock ages, its ability to produce eggs diminishes such that it is no longer economically feasible to keep the flock in lay. Producers have the option of sending birds to slaughter and bringing on a new flock. This choice causes problems such as the cost to raise the birds while receiving no income until they come into lay and the serious situation of disposal of the previous flocks.

Because of the growth of the broiler industry and its expansion into commercial areas previously reserved for spent hens, the market for retired layers is minimal. Whereas in earlier times producers might recoup a small sum of money for their old layers from soup manufacturers and similar industries, the current situation dictates that producers must absorb the cost of hen elimination and, on many occasions, are forced to transport birds long distances for the disposal. As a consequence, methods are continually sought to extend the effective laying life of a flock.

Induced molting remains an important management tool for the layer industry as a means to maximize the effective laying life of a flock. "Molting programs involve an estimated 75 to 80% of the commercial flocks in the U.S. At any point in time, 25 to 30% of the nation's layers are either in a molt or have been molted earlier…" (Bell, 2001). Considering that there is an estimated 256 million hens in the U.S., the number of hens molted annually would be 192 to 204 million. Approximately one-third of the profits of a flock is estimated to come from molted birds.

Although there are many procedures to molt hens, the primary method for molt induction is to remove feed until hens lose a certain percentage of body weight (USDA, 2000a). However, previous experimental studies have shown that this procedure significantly depresses the cell-mediated immune response in the hens (Holt, 1992a), and flow cytometric analysis of peripheral blood

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from molted and nonmolted hens demonstrated that the CD4+ T cells, the helper T-cell subset, are significantly decreased in molted hens (Holt, 1992b). An intact immune response is crucial for the health of the bird by providing protection against the wide array of microbial pathogens that attack laying flocks. By compromising that immune protection during situations such as induced molt, the flocks may be vulnerable to infection by a number of these organisms.

One organism in particular, *Salmonella enterica* serovar Enteritidis (SE), was studied extensively with regards to infection during molt. Molted hens shed more SE from their feces (Holt, 1993; Holt and Porter 1992a,b, 1993; Holt et al., 1994, 1995), had higher levels of SE in internal organs (Holt et al., 1995), remained infected for longer periods (Holt, 1995; Holt and Porter, 1992a), exhibited more pathology in the intestinal tract than their nonmolted counterparts (Holt and Porter, 1992a; Macri et al., 1997; Porter and Holt, 1993), and caused the recrudescence of a previous SE infection (Holt and Porter, 1993). The hens were more susceptible to SE infection—approximately 5×10^4 SE were necessary to infect 50% of fed hens, whereas less than 10 SE were necessary in hens undergoing molt via feed withdrawal (Holt, 1993).

Because infection can be initiated with so few organisms, SE will readily transmit horizontally to other molted hens in a simulated flock situation either through bird-to-bird contact (Holt and Porter, 1992b; Holt, 1995) or via the airborne route (Holt et al., 1998). A change in the dynamics of the SE infection was also observed during the molt. In normal-fed birds, SE resided primarily in the cecum, with some organisms found in the colon but little elsewhere. During the molt, however, SE are detected more extensively throughout the intestinal tract (Holt et al., 1995). Alterations in SE levels in the intestine during molt occur rapidly, within 48 h, indicating that other protective mechanisms besides specific immunity are affected by the molt procedure. Heterophils, part of the first line of defense against infection, have decreased activity during molt (Kogut et al., 1999).

The above results reflect the effects of induced molting on SE infection under experimental, controlled conditions and may not reflect what actually occurs in commercial field situations. Several studies followed this avenue of investigation. Research conducted by the USDA/APHIS SE Pilot Project showed that the production of eggs dramatically increased the first weeks following a molt (USDA, 1995). The USDA/APHIS NAHMS Layers 99 study found that there was a doubling of environmental SE numbers in molted versus nonmolted flocks (USDA, 2000b). Murase et al. (2001) showed that the levels of environmental salmonellae increased dramatically in commercial flocks following a molt. These results provide some indication that molting may exacerbate an SE problem in commercial houses, but, clearly, much more research is needed to determine how much of a problem actually exists and what management procedures, including the type of molt, may play a role in this situation.

If molting hens via long-term feed withdrawal creates SE problems within a flock, what strategies might be implemented to ameliorate the situation? Seo et al. (2000) found that administration of a regimen of the antibiotic enrofloxacin followed by a bacterial probiotic dramatically reduced the incidence of fecal shedding and internal organ contamination by SE in molted hens. More recently, Holt et al. (2002) showed that hens vaccinated with a live attenuated *Salmonella typhimurium* just prior to molt exhibited dramatic reduction in horizontal transmission of SE to hens exposed during molt. One or two aerosol doses of vaccine provided strong protection. Finally, peristalsis in combination with digesta provides a very effective cleansing mechanism to sweep potential pathogens out of the bowel.

By eliminating feed, this cleansing mechanism would be compromised, allowing a host of potential problem organisms to proliferate in the bowel. This was borne out in earlier studies by Freter (1955) and Formal et al. (1958) in rodents. Feeding hens a low-energy, low-calcium diet, in metered amounts, will put them into a molt (Rolon et al., 1993) and significantly reduce intestinal shedding and inflammation in hens challenged with SE (Holt et al., 1994). However, the necessity for administering metered amounts of feed decreased the utility of this molt program for commercial operations as it was labor intensive and would be difficult to ensure all birds received their allotted amount of feed. Hens fed ad libitum wheat middlings, a by-product of wheat processing, would be induced to molt and, when challenged with SE, exhibit significantly less SE shedding and internal organ contamination compared with hens molted via feed withdrawal (Seo et al., 2001). Other molt regimens using cracked corn or soybean hulls reduced SE problems to a certain extent but were much less effective than wheat middlings (Holt, data not shown). Such a regimen would be more acceptable by industry if production parameters paralleled those observed for feed withdrawal. Recent studies by Biggs et al. (2001) indicated that wheat middlings effectively put hens into a molt and the subsequent production postmolt were similar to those observed in hens subjected to long-term feed withdrawal. Preliminary studies by Kwon et al. (2001) using an alfalfa diet as well as Kubena et al. (2001) and Ricke et al. (2001) using high levels of zinc and low calcium/low zinc diets, respectively, showed promise, and it is hoped that other regimens will be tested for their usefulness as an alternative molt procedure.

The current paper provides a brief summary of what is known about molting and its potential to cause problems in laying hens. Clearly, much remains to be done, especially with regard to work in commercial operations. Questions such as Does molting via long-term feed withdrawal really exacerbate SE problems in flocks? If so, is there a specific length of time hens can remain off feed and not experience these problems? What about alternative days of feeding then fasting? Do management practices such as cage density, house size, manure disposal, or SE vaccination affect the problem?

Feeding hens wheat middlings worked well as an alternative molt procedure, and there are probably other feedstuffs that might work as well or better than the middlings in establishing an effective molt and limiting the SE problem. Hopefully, answers to these and other questions will be forthcoming and solutions will be found that are acceptable for producers and consumers alike.

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